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Short communication

The complete genome sequence of *Bacillus velezensis* strain GH1-13 reveals agriculturally beneficial properties and a unique plasmid

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ABSTRACT

The bacterial strain *Bacillus velezensis* GH1-13, isolated from rice paddy soil in Korea, has been shown to promote plant growth and have strong antagonistic activities against pathogens. Here, we report the complete genome sequence of GH1-13, revealing that it possesses a single 4,071,980-bp circular chromosome with 46.2% GC-content. The chromosome encodes 3,930 genes, and we have also identified a unique plasmid in the strain that encodes a further 104 genes (71,628 bp and 31.7% GC-content). The genome was found to contain various enzyme-encoding operons, including indole-3-acetic acid (IAA) biosynthesis proteins, 2,3-butanediol dehydrogenase, various non-ribosomal peptide synthetases, and several polyketide synthases. These properties are responsible for the promotion of plant growth and the biosynthesis of secondary metabolites. They therefore have multiple beneficial effects that could be applied to agriculture. Through curing, we found that the unique plasmid of GH1-13 has important roles in the production of phytohormones, such as IAA, and in shaping phenotypic and physiological characteristics. The plasmid therefore likely influences the biological activities of GH1-13. The complete genome sequence of *B. velezensis* GH1-13 contributes to our understanding of this beneficial strain and will encourage research into its development for agricultural or biotechnological applications, enhancing productivity and crop quality.

1. Introduction

The use of beneficial microorganisms is considered a safe and sustainable way for crop management practices to improve yield and quality (Fravel, 2005). Members of the *Bacillus* genus are widely used in agriculture due to their ability to promote plant growth and/or suppress plant pathogens (Lugtenberg and Kamilova, 2009). Over the last decade, *Bacillus velezensis*, a recently re-classified synonym of *B. methylotrophicus*, *B. amyloliquefaciens* subsp. *plantarum*, and *B. oryzicola* (Dunlap et al., 2016), has been intensively studied and used in the agricultural sector (Borriss, 2011). Some *B. velezensis* strains, primarily those isolated from the rhizosphere, have been shown to be beneficial to crops by promoting growth through the production of phytohormones, such as auxin and other volatile organic compounds (e.g. 2,3-butanediol) (Ryu et al., 2004). Additionally, these species can produce a wide array of secondary metabolites that protect plants from pathogenic infection (Lugtenberg and Kamilova, 2009).

Previously, Kim et al. (2016) have reported that the *B. velezensis* strain GH1-13 has multifunctional effects on plant growth through indole-3-acetic acid (IAA) production and also possesses strong antagonistic activities against pathogenic bacteria and fungi, such as *Fusarium fujikuroi, Rhizoctonia solani,* and *Xanthmonas oryzae*. These effects on plant pathogens are potentially mediated via the production of various secondary metabolites (Chen et al., 2009; Chowdhury et al., 2015). Although genomic features unique to *B. velezensis* GH1-13 are expected to be linked to growth promotion and pathogen antagonistic activity, these details are still unclear due to the small number of complete genomes for *B. velezensis* strains. Therefore, our complete genome sequence of GH1-13 provides a better understanding of the mechanisms involved in promoting plant growth and the biological control of plant diseases in eco-friendly agriculture. This has the potential to replace chemical fertilizers and pesticides, while enhancing productivity and crop quality.

2. Materials and methods

2.1. Isolation and DNA extraction

The *B. velezensis* strain GH1-13 was isolated from a rice paddy field in Goheung, Korea. Genomic DNA was extracted from cultured cells grown to stationary phase in tryptic soy broth (TSB) medium using a QIAamp DNA mini kit (Qiagen, USA), according to the manufacturer's protocols.

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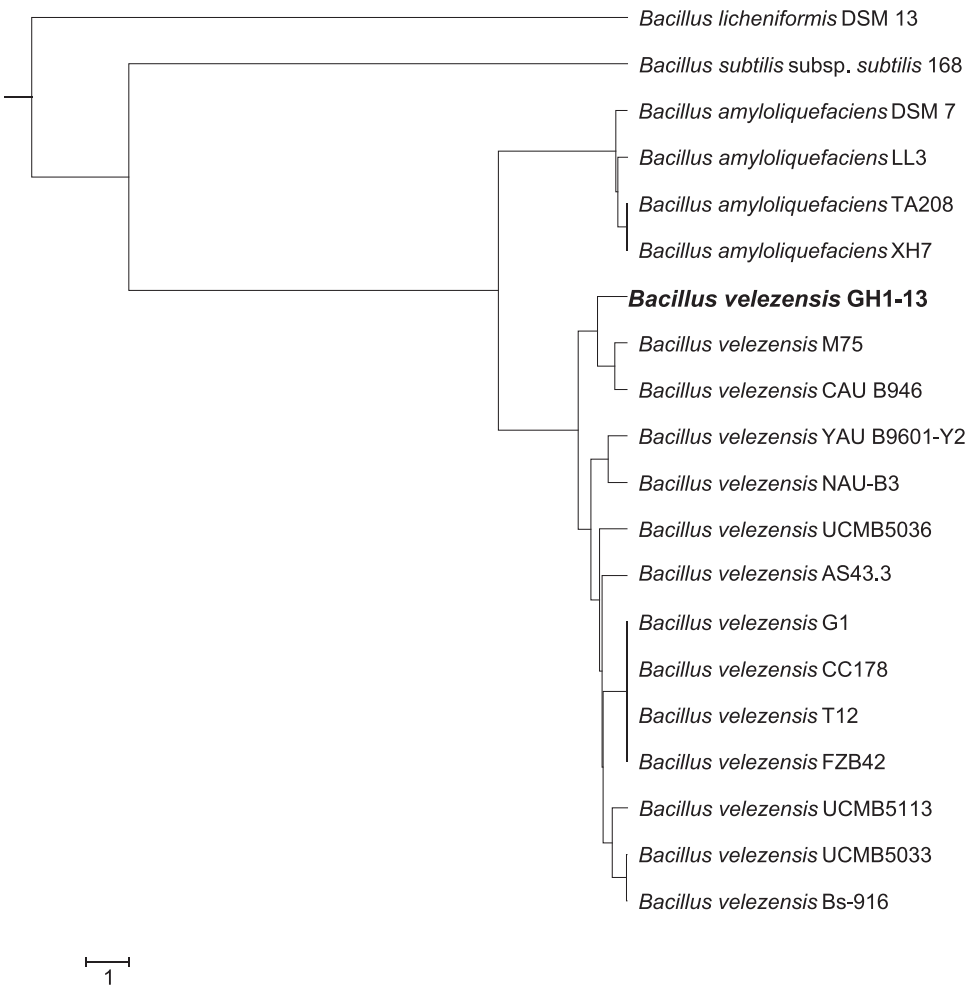

Fig. 1. Phylogenetic tree of *Bacillus velezensis* GH1-13, and its closest relatives, among publically available reference genomes. The tree was constructed with MEGA 6.0 using a neighbour-joining method, based on average nucleotide identity. The strain *Bacillus licheniformis* DSM 13 was used as an outgroup.

2.2. Genome sequencing, assembly and annotation

To investigate genomic features that associated with growth promotion and the antagonistic activities of GH1-13, the whole genome of the organism was sequenced with a 20-kb SMRTbell™ template library using Pacific Biosciences (PacBio) RSII Single Molecule Real Time (SMRT) sequencing at Chunlab (Seoul, Korea). The genome was assembled and annotated using previously detailed analytical procedures (Kim et al., 2017). Briefly, all of the obtained reads were assembled into a single circular chromosome, without gaps, using SMRT Analysis 2.3.0 software. Gene annotation was performed using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) and Rapid Annotation Subsystem Technology (RAST server; Aziz et al., 2008). Gene clusters related to the biosynthesis of secondary metabolites were predicted based on analysis with antiSMASH 3.0 (Weber et al., 2015) and BAGEL3 (van Heel et al., 2013). A phylogenetic tree was constructed using a neighbour-joining method, based on average nucleotide identity, with MEGA software (version 6.0).

2.3. Plasmid curing

The plasmid of *B. velezensis* GH1-13 was cured by elevated growth temperatures following a previously described protocol by Carlton and Brown (1981), with slight modifications. Briefly, the GH1-13 was incubated overnight at 28 °C (150 rpm) in sterile 5 mL aliquots of tryptic soy broth (TSB) to the stationary phase (optical density$_{600nm}$: approximately 1.5). After incubation, cell culture was diluted 100-fold in TSB and then re-incubated at 45 °C (150 rpm) overnight. This step was successively repeated five times to obtain a plasmid cured strain. Serial dilutions were created and plated on tryptic soy agar (TSA) to obtain single colonies. These were then incubated at 28 °C overnight. Total DNA was extracted using a QIAamp DNA mini kit (Qiagen, USA), according to the manufacturer's protocol. The extracted total DNA was amplified by PCR in both GH1-13 and potentially cured strains using specific primer sets based on the chromosome and plasmid sequences for the GH1-13 strain (chromosome, amplicon size 232 bp, GHSC-F1: 5′-CGCCGAACGGAAAGTAGA-3′, GHSC-R1: 5′-GAGAGCGGTCAAAAGATGG-3′; plasmid, amplicon size 217 bp, GHSP-F1: 5′-GTACATTTTTCCATTTTATTTCCCCTCCTT-3′, GHSP-R1: 5′-GGTGAATAAAATGGCGAAAACAACAGTAA-3′). For PCR, the conditions were an initial step at 95 °C for 5 min and then 32 cycles of 45 s at 95 °C, 45 s at 60 °C, and 45 s at 72 °C, with a final 10 min incubation at 72 °C. The presence or absence of amplicons was confirmed using a 1.2% agarose gel to reveal which strains were plasmid cured (termed GH1-13cp) (Fig. S1).

2.4. Measurement of IAA production potential

Indole acetic acid (IAA) production was determined using a colorimetric analysis (Gordon and Weber, 1951) in the presence of L-tryptophan (0.1% wt V^{-1}) as a IAA precursor. Bacteria were grown in tryptone soy broth (TSB) at 28 ± 2 °C for 24 h with continuous shaking at 150 rpm. After the shaking, supernatant was collected by centrifugation at 4000*g* for 15 min. IAA was detected by mixing 1 mL of Salkowski reagent (1 mL 0.5 M $FeCl_3$ and 50 mL concentrated perchloric acid (35%)) with 500 µL of supernatant, and allowed to react at room temperature for 30 min. IAA production was confirmed by pink colour development as quantified at 530 nm. A standard curve was prepared using concentrations of 0, 25, 50, and 100 mg IAA L^{-1}. Blanks